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Exhaustive Extraction of Bioactive Components from *Sargassum cristaefolium* Brown Seaweed: Antioxidant Potential and Bioactivity

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ABSTRACT

Bioactive components are essential ingredients of functional foods, supplements, pharmaceuticals, etc. *Sargassum cristaefolium* brown seaweed, as an Indonesian marine resource, provides promising bioactive components. The present study was to extract the total bioactive components of *S. cristaefolium* with a microwave instrument. The extraction method was done serially using different polarity solvents (1st-stage: non-polar, 2nd-stage: semi-polar, Final-stage: polar). Yielded extracts were analyzed for bioactive compounds, functional groups, total phenolic and flavonoid, and antioxidative activities. The results showed that all staged extractions obtained bioactive compounds with various characteristics. However, the 2nd-stage extract was superior, and it exhibited the highest total phenolic and flavonoid (17.53±0.78 mg GAE/g, 72.64±3.01 mg QE/g), the richest volatile bioactive compounds (neophytadiene and phytol were dominant), and the predominant bioactive compound of antioxidative (oleoylethanolamide). Their functional groups confirmed the structure of antioxidative phenolic molecules: C—C stretching skeleton (phenyl/aromatic core), C—H stretching, C—H bending, and O—H stretching. The strongest primary (1439.84±63.02 μg/ml) and secondary (389.73±16.71 μg/ml) antioxidant activities were presented by the 2nd-stage extract. The efficiency of MAE and the potential of *S. cristaefolium* were promising for developing functional foods and pharmaceuticals that relate to antioxidants in the future.

1. Introduction

Brown seaweed, especially the *Sargassum cristaefolium* species, is an abundant natural resource in Indonesian waters (Prasedya *et al.* 2021b) and is known to be rich in bioactive compounds that have the potential to be developed in the necessities of functional foods, health supplements, herbal products, and pharmaceutical industries. Bioactive compounds, e.g., phenolics, terpenoids, alkaloids, saponins, tannins, and steroids, are important for health. They have a significant role in the prevention and treatment of various chronic diseases such as antioxidants, anticancer, anti-inflammatory, antidiabetic, cardioprotective, etc. (Payghami *et al.* 2015). The increasing requirement for safe and effective natural health products encouraged further work to extract bioactive compounds efficiently.

In recent decades, attention has been paid to the development of efficient technologies for extracting bioactive components. The extraction of bioactive components from natural materials has developed rapidly using various techniques, one of which is microwave technology. Microwaves, as an extraction technology, had advantages in terms of time and energy efficiency, as well as the ability to increase the yield and purity of the extracted compounds (Amarante *et al.* 2020). Lourenço-Lopes *et al.* (2023) add that this technology could provide advantages such as faster process time, increased yield, and natural loss reduction of extracted bioactive components than conventional methods.

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In the present study, microwave technology was applied to extract the total bioactive components of *Sargassum cristaefolium* brown seaweed based on the polarity of the solvent used sequentially, namely non-polar, semi-polar, and polar. The bioactive component contents were comprehensively analyzed to know their antioxidative activities and bioactivity or health benefits. Accordingly, their potential application for the requirement of pharmaceuticals and functional food can be more promising in the future.

2. Materials and Methods

2.1. Material

The main material for the present study was *Sargassum cristaefolium* brown seaweed obtained from Indonesian waters at Bimorejo Beach, Banyuwangi Regency, East Java, Indonesia (-7.94000066216285, 114.42341937569608). This seaweed was collected from the beach in fresh condition and then washed with fresh water to remove sand, salts, and other interference dirt before being dried at a low temperature, viz. 40°C until 10% moisture content as dry matter according to the Susilo *et al.* (2022) method. After drying, *Sargassum cristaefolium* was crushed into a fine powder (50 mesh) using a conventional grinding machine. *Sargassum cristaefolium* powder was then used for the extraction process.

Chemicals: *n*-hexane, ethyl acetate, methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), ascorbic acid, ferrous chloride, ferrozine, ethylenediaminetetraacetic acid (EDTA), folin-ciocalteu reagent, sodium carbonate, gallic acid, quercetin, natrium nitrite, aluminum chloride hexahydrate, sodium hydroxide, acetonitrile, formic acid, and potassium bromide used an absolute grade purchased from Merck Group (Darmstadt, Germany).

2.2. Extraction Process Serially

The extraction of bioactive components in *Sargassum cristaefolium* was carried out using microwave-assisted extraction (MAE) (Susilo *et al.* 2023a), which has been proven to be efficient and quick in separating bioactive compounds from natural materials. The extraction process was employed serially with different polarity solvents consisting of *n*-hexane, ethyl acetate, and methanol in consecutive. The *n*-hexane, ethyl acetate, and methanol had different relative polarities based on the empirical solvent parameter E^{N}_{T} value (0.009, 0.228, and 0.762, respectively, indicating low polarity to high polarity) (Reichardt and Welton 2010), so they are chosen to correspond on comprehensive polarity characteristics of phytochemicals i.e. non-polar/low polarity, semi-polar/medium polarity, and polar/high polarity. As (Susilo *et al.* 2023a, 2023b, 2024) studies, solvents with different polarity characteristics (non-polar up to polar) were proven to extract phytochemicals of various polarities completely (such as terpenoid, phenolic, alkaloid, saponin, and amino acid derivatives).

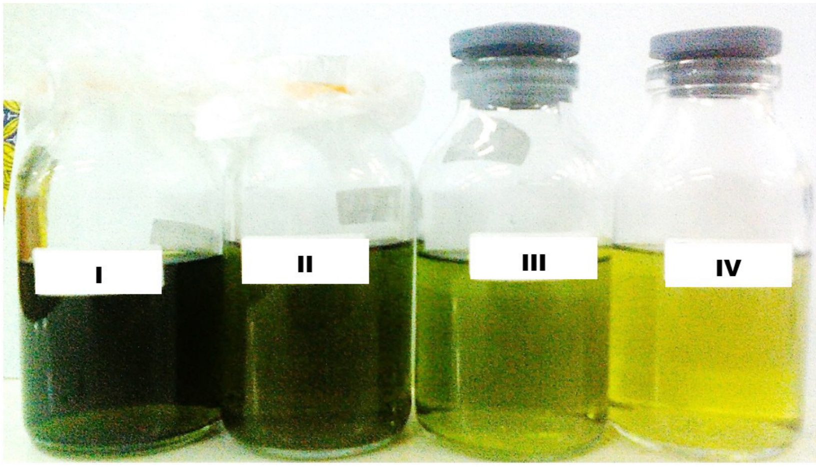
Initially, *Sargassum cristaefolium* powder was weighed as much as 2.5 g (powder/solvent ratio 1:20 g/ml), and each solvent volume was put into an extraction container in an MAE machine. Specific MAE (Multiwave PRO-Anton Paar) parameters were set as follows: magnetron frequency 2455 MHz, temperature 50°C, power 524 W, pressure 4.2 bar, pressure rate 0.5 bar/s, rotor speed 3 rpm, air humidity 66%, sample stirrer at high speed, and for 5 minutes run time. 1st-stage extraction with non-polar/*n*-hexane solvent, then after dried powder residues, were re-extracted. 2nd-stage extraction with semi-polar/ethyl acetate solvent, then after dried powder residues, were re-extracted for the final stage. Final-stage extraction with polar/methanol solvent. The powder residue drying of each extraction stage was conducted with an oven (Memmert) at 40°C until a constant mass was reached or the solvent residues were free. Figure 1 exhibits the extracts obtained from extraction process repetitions up to 4 times at each extraction stage. These are to ensure that the bioactive components in Sargassum cristaefolium have been extracted exhaustively. In the extraction process repetitions, all used extraction procedures and MAE machine settings were equal. Finally, all extraction results (Figure 1) were evaporated with a rotary evaporator (Heidolph Korea Ltd.) to free the solvents so, yielding the pure extracts, namely 1st-stage extract (non-polar/*n*-hexane), 2nd-stage extract (semi-polar/ethyl acetate), and final-stage extract (polar/methanol). The extracts were analyzed in the next step.

As the control extract, distilled water (pH 7) substituted the organic solvents in the extraction process with both the method and MAE settings, which were equal.

2.3. Analysis of Bioactive Compounds

All yielded extracts from each stage were analyzed and identified as the content of their bioactive compounds. The analysis instruments were employed, including Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) (Thermo

1st-stage extract by non-polar solvent



2nd-stage extract by semi-polar solvent

Final-stage extract by polar solvent

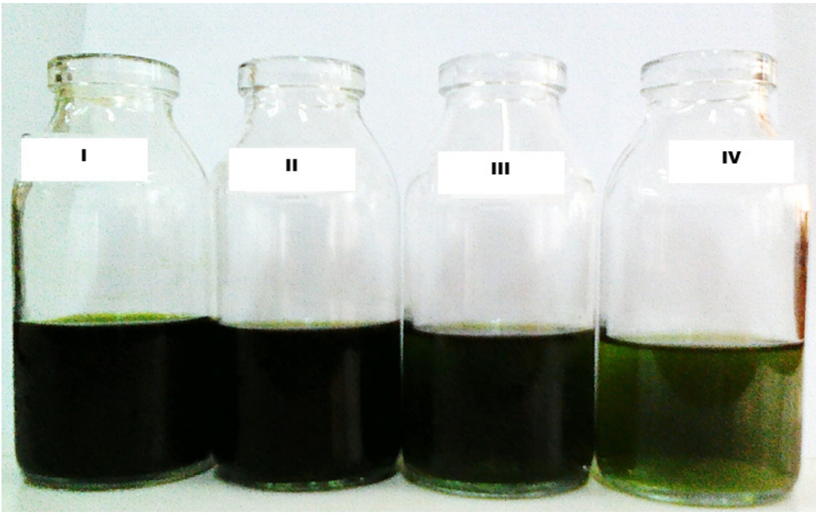


Figure 1. The serial extraction process with MAE obtains the extracts up to 4-time repetitions on each stage (1st-stage, 2nd-stage, and final-stage extracts). Explanation: the final repetition (IVth) on each stage possesses the clearest colour, it justifies that bioactive components have been extracted maximally since the clear colour is the solvent's own. Additionally, the extraction process repetitions are sufficient up to 4 times to save the employing cost of solvents. Thus, the extraction process is finished at IVth repetition of each stage extract

Scientific Q Exactive Ultimate 3000 by Thermo Fisher Scientific Inc.-Waltham, Massachusetts, USA.), Gas Chromatography-Mass Spectrometry (GC-MS) (Shimadzu GCMS-QP2010 Ultra by Shimadzu Corporation-Tokyo, Japan.), Fourier-transform infrared spectroscopy (FTIR) (FTIR8400S by Shimadzu Corporation-Tokyo, Japan.), and Microplate Reader (SPECTROstar Nano by BMG LABTECH-Ortenberg, Germany).

2.3.1. Quantification of Total Phenolic and Total Flavonoid

The total phenolic content of *Sargassum cristaefolium* extracts was quantified by analysis following the Susilo *et al.* (2023a) method. The method is similar without any modifications, with the result expressed as mg GAE/g.

The total flavonoid content was determined using the Deng *et al.* (2015) method with slight modifications. The reduction of test liquid volumes was modified to conform to the used analysis instrument, which is the Microplate Reader in the current study. In the Costar 96-well plate flat bottom, extracts/standard (75 µL) and $NaNO_2$ 5% (22.5 µL) were dropped, the mixture was shaken and then incubated for 5 min at ±25°C. Thereafter, $AlCl_3$ 10% (22.5 µL) was added with similar both shaking and incubating again. NaOH 1M (150 µL) was added, and then the overall mixture was shaken and incubated (15 min, ±25°C) for the final reaction. The absorbance of the reaction mixture was read using the Microplate Reader at 415 nm. The total flavonoid content in the extracts was calculated based on the quercetin standard curve and expressed in mg QE/g.

2.3.2. Analysis of Volatile Bioactive Compounds

The volatile bioactive compounds on Sargassum cristaefolium extracts were identified using GC-MS with qualitative analysis. Majchrzak *et al.* (2018) noted that either qualitative or quantitative analysis of the volatile phytochemical compounds in the natural products could be determined using GC-MS by the GC-MS principles to compare the detected spectra of samples with the compound libraries. One µl diluted extract solution in the methanol was injected directly without any derivatization. Helium was a carrier gas in the mobile phase. The total flow rate was 54.2 ml/min with the oven temperature program for retention time: initial rate (150°C, 1 min hold time), then up rate (15°C/min, 220°C, 22.33 min hold time, up to 28 min total run time), and pressure in 13.32 psi. The capillary column as a stationary phase used HP-5MS 5% Phenyl Methyl Siloxane (Agilent 19091S-433) with the size: 325°C max, 30.0 m × 250 µm × 0.25 µm, and average velocity: 38 cm/s. The detected chromatogram spectra were then analyzed by NIST02 and WILEY275 libraries. The PubChem page and the scientific evidence searched for the bioactivity or potential health benefits of the detected volatile compounds.

2.3.3. Analysis of Metabolomic Bioactive Compounds

Various metabolomic bioactive compounds contained in *Sargassum cristaefolium* extracts were identified with LC-HRMS using the non-targeted screening method (Susilo *et al.* 2020). LC-HRMS, as an advanced method, was completely recognized for bioactive compound identification. It possesses high mass resolution and high calculation on the mass accuracy of each detected single compound for LC-HRMS analysis of the *Sargassum cristaefolium* extracts, fully equaled the Susilo *et al.* (2020) method without any modifications. After all compounds were identified, their bioactivity or potential health was searched using the PubChem page and the scientific evidence.

2.3.4. Analysis of Functional Groups

FTIR analysis determined the functional groups of *Sargassum cristaefolium* extracts to confirm the elucidation of its bioactive compound structures. Briefly, using the Moubayed *et al.* (2017) method, 0.1 g of *Sargassum cristaefolium* extracts and 0.1 g tablet of potassium bromide (KBr) were mixed. Thereafter, the mixture was formed into pellets or salt discs with 3 mm diameter through a strong pressing process. The pellets were inserted into the FTIR instrument, which has been set with wavelength 4000-400 cm^{-1} for spectra recording. Finally, data results were obtained from standard software possessed by the instrument.

2.4. Evaluation of Antioxidant Capacity

Sargassum cristaefolium extracts were evaluated for their antioxidant capacity, both primary antioxidants on free radical scavenging and secondary or preventive antioxidants on chelating of pro-oxidant ferrous ions. The primary antioxidant was tested on scavenging DPPH free radicals by following the Susilo *et al.* (2023b) method similarly, without any modification applied.

Meanwhile, the preventive antioxidant on chelating of pro-oxidant ferrous ions used the Končić *et al.* (2011) method. Costar 96-well plate flat bottom, the extracts solution (150 µL) in the methanol and 0.25 mM $FeCl_2$ solution (50 µL) were added and subsequently incubated for 5 min. Afterward, 1.0 mM ferrozine solution (100 µL) was added and incubated for 10 min at room temperature, then absorbance was read at 545 nm. As a control, a reaction mixture in methanol without the samples (150 µL) instead of the sample solutions. EDTA was applied as the chelating standard. The chelating activity of pro-oxidant ferrous ions was determined using the equation [1] below, and the value was expressed in IC_{50}, viz., the concentration to chelates 50% of Fe^{2+} ions.

$$\text{Chelating activity} = \frac{\text{Abs.control - Abs.Sample}}{\text{Abs.Sample}} \times 100 \qquad \text{Eq. [1]}$$

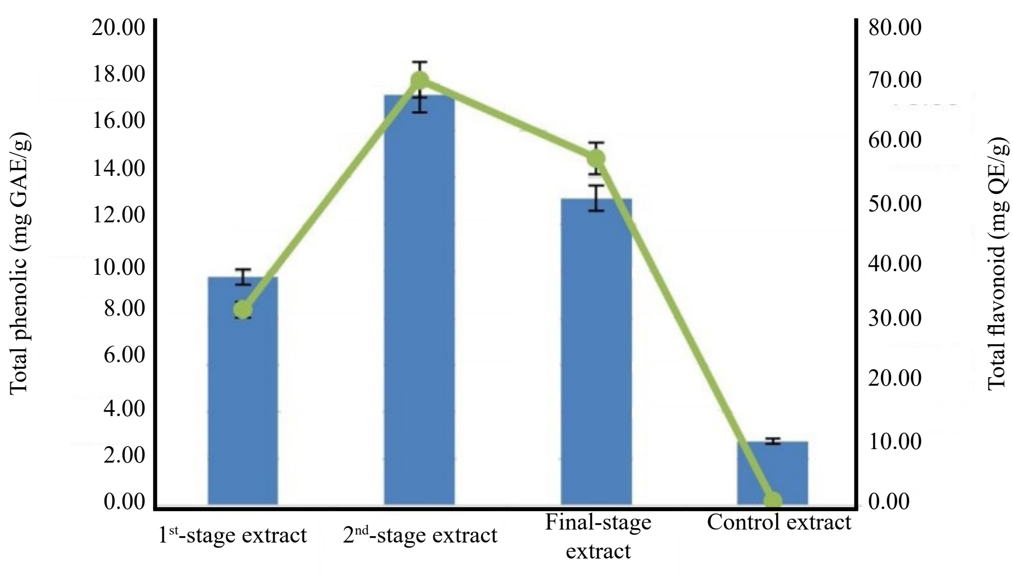
2.5. Statistical Calculation

Quantitative data of three replications were presented on average ± standard deviation. Significant analysis used a group-randomized design with analysis of variance (ANOVA) was worked by Minitab 18 software, hereafter Tukey test at $p<0.05$ to present a significant difference.

3. Results

3.1. Total Phenolic and Total Flavonoid Content

Figure 2, the content of the total phenolic and flavonoid in Sargassum cristaefolium brown seaweed extracts revealed variations across the three extraction stages. The second-stage extract (semi-polar solvent) exhibited the highest total phenolic (17.53±0.78 mg GAE/g) and flavonoid (72.64±3.01 mg QE/g) content significantly at $p<0.05$. Conversely, the first-stage extract was the lowest significantly (total phenolic = 9.76±0.33 mg GAE/g, total flavonoid = 33.44±1.36 mg QE/g). The results indicated a more selective phenolic and flavonoid compound of *Sargassum cristaefolium* solubility in semi-polar environments. The control extract had a lower total phenolic (2.75±0.11 mg GAE/g) and flavonoid content (0.78±0.03 mg QE/g) than the extracts from organic solvents since using distilled water in the extraction process, which is suitable for the polarity characteristic of those compounds. Meanwhile, several previous research reports that *Sargassum cristaefolium* extraction with other technologies, either conventional or advanced (Table 1), exhibits total phenolic and flavonoid, as



	1st-stage extract	2nd-stage extract	Final-stage extract	Control extract
Total phenolic	9.76	17.53	13.11	2.75
Total flavonoid	33.44	72.64	59.26	0.78

Figure 2. Total phenolic and total flavonoid content of *Sargassum cristaefolium* brown seaweed extracts

Table 1. Comparison with previous research for bioactive component extraction methods of *Sargassum cristaefolium* brown seaweed

Extraction method	Process	Solvent	Total phenolic (mg GAE/g)	Total flavonoid (mg QE/g)	IC_{50} of DPPH antioxidant (μg/ml)	Research
Maceration	1:5 (w/v), 48 h	Ethanol	66.13	-	737.30	(Prasedya *et al.* 2021b)
Maceration	40°C, 3 h	Methanol:Water (1:1)	*0.80	-	-	(Lann *et al.* 2012)
Cold maceration	1:10 (w/v), 30 min, sample powder size <45 μm	Ethanol	43.27	70.27	202.70	(Prasedya *et al.* 2021a).
Sonication	15 min, sample powder size of 40 μm, centrifugated to separate the lipophilic extract	Chloroform : Methanol : Water (2:1:0.5)	-	-	206.70	(Sunarwidhi *et al.* 2023)
Maceration	1:10 (w/v), 24 h	Ethanol 96%	≈10	≈500	-	(Sunarwidhi *et al.* 2022)
UAE	1:10 (w/v), 30 min, 30 kHz	Ethanol 96%	≈45	≈600	-	(Sunarwidhi *et al.* 2022)
Maceration	1:10 (w/v), room temperature (29±2°C), 24 h	Methanol	2.07	-	-	(Saraswati *et al.* 2020)
Sonication	1:20 (w/v), 30 min at 55°C, and after sonication then shaken for 4 h (300 rpm)	Methanol	1.27	**17.10	-	(Silva *et al.* 2022)
MAE	1:20 (w/v), 50°C, 5 min, sample powder size of 297 μm, 2455 MHz	*n*-hexane, ethyl acetate, and methanol, serially	9.76 - 17.53	33.44 – 72.64	2520.75 – 1439.84	Present research

*:expressed as mg phloroglucinol equivalent/g, **: expressed as mg rutin equivalents/g, -: not analyzed, UAE: ultrasonic-assisted extraction, MAE: microwave-assisted extraction

well as antioxidative capacity that varies. Case by case depended on the extraction process, including the solvents used, extraction time, temperature, solvent/sample ratio, and material size. Nevertheless, MAE technology could extract Sargassum cristaefolium components within a short time, and its extract yield efficiency is competitive.

3.2. Abundance of Bioactive Compounds

3.2.1. Bioactive Compounds Reported by GC-MS

The GC-MS analysis identified multiple volatile bioactive compounds contained in the three extraction stages of *Sargassum cristaefolium* brown seaweed. Table 2 summarizes these findings, including their chemical structures, molecular weights, and relative percentages. The second-stage and final-stage extracts contained a higher abundance of volatile bioactive compounds (a total of 9 compounds). Notable compounds of the 2nd-stage extract, i.e., neophytadiene (7.45%), phytol (7.45%), and l-(+)-ascorbic acid 2,6-dihexadecanoate (4.64%), which are associated with antioxidant, anti-inflammatory, anticancer, cardioprotective, etc., properties. Notable compounds of the final-stage extract included hexyl cinnamic aldehyde (10.90%), 9-tricosene (7.30%), and 1-hexacosene (4.17%), which are associated with antioxidant, anti-obesity, antimicrobial activities, neuroprotective, etc., activities. Meanwhile, the 1st-stage extract had the lowest abundance of volatile bioactive compounds (total = 5 compounds) with notable compounds i.e. phenol, 2,4-bis(1,1-dimetiletil) (5.84%), its bioactivity highlighting more antimicrobial potential.

3.2.2. Bioactive Compounds Reported by LC-HRMS

LC-HRMS analysis provided a detailed profile of metabolomic bioactive compounds, as shown in Table 3. The extraction of *Sargassum cristaefolium* brown seaweed successfully separated metabolomic compounds based on the used solvent polarity. The total metabolomic bioactive compounds of the 1st-stage were more than the final stage and more than the 2nd-stage extracts successively (12, 10, and 7 compounds, respectively). The 1st-stage extract predominantly contained tolbutamide, 5-fluoro emb-fubinaca, and choline bioactive compounds, with

Table 2. Volatile bioactive compounds determined by GC-MS on *Sargassum cristaefolium* extracts

Bioactive compounds	Bioactivity/health beneficial reports	Formula	MW (g/mol)	RT (min)	% Relative		
					1st-stage extract	2nd-stage extract	Final-stage extract
Phenol, 2,4-bis(1,1-dimetiletil)	Antioxidant, anti-inflammatory, and whitening Effects (Lee *et al.* 2007) Neurodegeneratif protections (Kim *et al.* 2017) Anti-cancer (cytotoxic activity) (Golam Mostofa *et al.* 2021) Antidiabetes (T and Vigasini 2021) Antibacteria (Gupta *et al.* 2021) Antifungal (Ren *et al.* 2019; Devi *et al.* 2021) Antiviral (Sharaf 2020) Anti-protozoal parasite (Malekhayati *et al.* 2024)	$C_{14}H_{22}O$	206.32	4.088	5.84	1.41	1.21
Isololiolide	Anticancer (Vizetto-Duarte *et al.* 2016; Aïssaoui *et al.* 2019) Anti-protozoal parasite (Lima *et al.* 2019) Anticholinesterase for Alzheimer's disease (Xu *et al.* 2022) Anti-inflammatory (Huang *et al.* 2024) Antioxidant (Ratnayake *et al.* 2013; Rengasamy *et al.* 2019) Antitubercular activity for TBC (Zhao *et al.* 2014)	$C_{11}H_{14}O_2$	178.23	5.888	-	2.26	-
Neophytadiene	Antioxidant (Cheng *et al.* 2015; Bryology *et al.* 2024) Anti-inflammatory, cardioprotective (Bhardwaj *et al.* 2020) Anticholinesterase (Olasehinde *et al.* 2021) Neurodegenerative protection (Gonzalez-Rivera *et al.* 2023)	$C_{20}H_{38}$	278.52	6.351	-	7.45	-
Phytol	Antioxidant (Okpala *et al.* 2022; Onanuga and Okpala 2022) Hepatoprotective activity (Gupta *et al.* 2019) Antimicrobial (Pejin *et al.* 2014b) Anti-cholinesterase (Olasehinde *et al.* 2021) Anti-tumor malignant (Thakor *et al.* 2016) Neuroprotective on neurodegenerative disease (Vahdati *et al.* 2022) Anti-inflammatory (Silva *et al.* 2014) Immunostimulant (Hoseini *et al.* 2021) Anticancer (Pejin *et al.* 2014a) Anxiolytic, antinociceptive, antianxiety (Islam *et al.* 2018)	$C_{20}H_{40}O$	296.53	6.351	5.52	7.45	-
1-Hexadecene	Antimicrobial, antioxidant (Mou *et al.* 2013)	$C_{16}H_{32}$	224.42	4.562	-	0.67	-
9-Tricosene	Antifungal (Gołebiowski *et al.* 2015; Aswani *et al.* 2023)	$C_{23}H_{46}$	322.61	5.974	2.47	1.15	7.30
Cyclotetracosane	Antimicrobial (Makajanma *et al.* 2020) Antioxidant, anticancer (Rahamtulla *et al.* 2023)	$C_{24}H_{48}$	336.63	7.334	-	2.93	-

Table 2. Continued

Bioactive compounds	Bioactivity/health beneficial reports	Formula	MW (g/mol)	RT (min)	% Relative		
					1st-stage extract	2nd-stage extract	Final-stage extract
l-(+)-Ascorbic acid 2,6-dihexadecanoate	Antioxidant (Younes *et al.* 2021) Antimicrobial, antitumor (Karthikeyan *et al.* 2014) Anticancer (Bahrun *et al.* 2023)	$C_{38}H_{68}O_8$	652.95	7.763	-	4.64	-
cis-9-Hexadecenal	Antifungal (Hoda *et al.* 2019) Anti-inflammatory (Alves *et al.* 2021) Antioxidant, antibacteria (Gahtori *et al.* 2024)	$C_{16}H_{30}O$	238.41	10.203	-	2.47	-
1-Nonadecene	Antioxidant, antimicrobial (Heng *et al.* 2020)	$C_{19}H_{38}$	266.50	4.562	0.80	-	-
1-Hexacosene	Neuroprotective (Nmeazi *et al.* 2024)	$C_{26}H_{52}$	364.69	7.768	2.90	-	4.17
Triacetin	Antioxidative, immunomodulatory (Erukainure *et al.* 2016) Antiobesity (Islas-Garduño *et al.* 2023)	$C_9H_{14}O_6$	218.20	2.916	-	-	0.57
Lilial	Antioxidant, antibacteria (Esmaeili and Jafarzadeh 2016) Anticancer (Charles and Darbre 2009)	$C_{15}H_{20}O$	204.30	4.265	-	-	0.92
Methyl dihydrojasmonate	Anti-tumor, anticancer (Yehia *et al.* 2017) Antileukemia (Abou-Elnour *et al.* 2024) Anti-fatigue (Nishimura *et al.* 2024) Skin rejuvenation (Olejnik *et al.* 2018)	$C_{13}H_{22}O_3$	226.31	5.088	-	-	3.65
Hexyl cinnamic aldehyde	Antioxidant, antimicrobial (Sova 2012)	$C_{15}H_{20}O$	216.32	5.796	-	-	10.90
17-Pentatriacontene	Antiinflammatory, anticancer, antibacterial, antiarthritic (G *et al.* 2018)	$C_{35}H_{70}O$	490.93	5.991	-	-	3.19
Hexatriacontane	Anti-inflammatory (Esmat *et al.* 2020) Antimicrobial (Xuanji *et al.* 2016)	$C_{36}H_{74}$	506.97	8.380	-	-	1.35
1-Docosene	Antioksidant, antibacterial (Park *et al.* 2024)	$C_{22}H_{44}$	308.58	9.986	-	-	3.92
Total					Contains 5 bioactive compounds	Contains 9 bioactive compounds	Contains 9 bioactive compounds

-: not present, MW: molecular weight, RT: retention time

their bioactivities such as neuroprotective, anti-angiogenesis, hypoglycemic effects, etc. The final stage extract had dominant bioactive compounds, i.e., tolbutamide, betaine, and valpromide, with bioactivities including anti-diabetes, antioxidant, anti-inflammatory, neuroprotective, anticancer, antiepilepsy, etc. In the 2nd-stage extract, the bioactive compounds of tolbutamide, oleoylethanolamide, and R-Palmitoyl-(2-methyl) ethanolamide were predominantly. The result revealed the advantage of serial extraction with different polarity solvents in maximizing the extracted compound diversity.

3.3. Identification of Functional Groups

FTIR spectra analysis confirmed the presence of functional groups characteristic of bioactive compounds in *Sargassum cristaefolium* extracts. These functional groups confirmed the bioactive compound profiles determined by GC-MS, LC-HRMS, phenolic, and flavonoid. All extract stages (1st-stage, 2nd-stage, and final-stage); the identified functional groups including C-H in-plane bending (phenyl ring) at 950-1250 cm^{-1}, C-O stretching and O-H in-plane bending coupled (phenol) at 1310-1410 cm^{-1}, C-C stretching skeletal (phenyl nucleus) at ≈1450 cm^{-1}, and C-H

Table 3. Metabolomic bioactive compounds determined by LC-HRMS on *Sargassum cristaefolium* extracts

Bioactive compounds	Bioactivity/health beneficial reports	Formula	MW (g/mol)	RT (min)	Area (Max.)		
					1st-stage extract	2nd-stage extract	Final-stage extract
Tolbutamide	Anti-diabetes (Chakraborty *et al.* 2017)	$C_6H_{11}N_3O_2$	156.02	1.097	316,855,563	2,044,609,839	3,063,470,413
Choline	Essential nutrient (Zeisel and Da Costa 2009) Neuroprotective (Blusztajn *et al.* 2017)	$C_5H_{14}NO^+$	101.99	0.944	9,918,837	-	-
Oleamide	Anti-inflammatory (Ameamsri *et al.* 2020)	$C_{18}H_{35}NO$	281.26	16.493	8,501,082	8,141,256	4,104,772
5-Fluoro emb-fubinaca	Anti-angiogenesis (AL-Eitan and Kharmah 2024)	$C_{24}H_{39}O_2P$	390.27	18.676	17,484,928	-	1,883,392
α-Eleostearic acid	Antioxidant (Saha and Ghosh 2009) Anticancer (Grossmann *et al.* 2009) Anti-tumor (Tsuzuki *et al.* 2004)	$C_9H_{36}N_8O_2$	278.22	14.903	1,858,848	331,046	-
Amobarbital	Antioxidant, osteoartritis protection (Quarterman *et al.* 2022)	$C_{11}H_{18}N_2O_3$	198.16	13.558	1,271,891	-	-
Oleoylethanolamide	Antiobesity, analgesic effect (Thabuis *et al.* 2008) Anti-inflammatory, immunomodulatory, antioxidant (Ghaffari *et al.* 2020)	$C_{20}H_{39}NO_2$	307.28	16.993	658,780	498,731,199	706,588
Sinefungin	Anti-infection (Nolan 1987) Antiviral (Kuroda *et al.* 2019)	$C_{12}H_{21}PS$	228.11	10.628	388,025	-	-
Neohesperidin dihydrochalcone	Anti-oxidative, anti-inflammatory, hepatoprotective (Hu *et al.* 2014) Neuroprotective for Alzheimer disease (Chakraborty *et al.* 2018)	$C_{26}H_{22}O_3S$	414.13	19.908	270,903	-	-
Paclitaxel	Anticancer (Sati *et al.* 2024)	$C_8H_{10}N_2O_3$	182.07	9.697	194,153	-	-
Erucamide	Antidepressant, anti-anxiety (Li *et al.* 2017) Alzheimer disease protection (Kim *et al.* 2018)	$C_{19}H_{39}N_2OP$	342.28	8.919	169,357	-	-
Ascorbyl palmitate	Antioxidative (Imran *et al.* 2024)	$C_{17}H_{36}N_2S$	300.26	17.541	341,373	-	665,191
R-Palmitoyl-(2-methyl) ethanolamide	Analgesic, anti-inflammatory (D'aloia *et al.* 2020)	$C_{19}H_{39}NO_2$	283.28	19.317	-	9,506,593	9,066,148
2-(14,15-Epoxyeicosatrienoyl) glycerol	Anti-inflammatory, antioxidant (Zeng *et al.* 2024)	$C_{22}H_{43}NO_2P_2$	415.28	12.853	-	5,207,260	3,924,810
Ethylene glycol tetraacetic acid (EGTA)	Antioxidative (Song *et al.* 2014)	CHN_5S	114.99	1.016	-	1,494,797	-

Table 3. Continued

Bioactive compounds	Bioactivity/health beneficial reports	Formula	MW (g/mol)	RT (min)	Area (Max.)		
					1st-stage extract	2nd-stage extract	Final-stage extract
Betaine	Antioxidant, anti-inflammatory, anti-nekrotik, neuroprotective, anticancer (Arumugam *et al.* 2021)	$C_5H_{11}NO_2$	118.00	0.929	-	-	341,146,295
Valpromide	Antiepilepsy, antipsikotic (Bialer 1991)	CHN_5S	115.97	0.966	-	-	20,256,255
Monoolein	Reduces the risk of chronic lung disease (Chan *et al.* 2022) Antitumor (Rongpan *et al.* 2558)	$C_{21}H_{41}O_2P$	356.28	13.275	-	-	556,438
Total					Contains 12 bioactive compounds	Contains 7 bioactive compounds	Contains 10 bioactive compounds

-: not present, MW: molecular weight, RT: retention time

stretching (aromatic) at 3000-3100 cm^{-1}, was detected across all extract stages to indicate phenolic structures. Flavonoid structures were detected by their specific functional groups, i.e., substituted benzene ring (C-H and C-C bending) at 675-900 cm^{-1}, C-H stretching (aromatic) at 3000-3100 cm^{-1}, and O-H stretching bonded (polymeric hydroxy) at 3200-3400 cm^{-1}. The functional groups ofalkenes (C=C stretching at 1600-1650 cm^{-1}) andalkanes (C-H stretching at 2843-2955 cm^{-1}) were detected in all extracts as well. They confirm the presence structure of hydrocarbon compounds such as oleamide, oleoylethanolamide, erucamide, monoolein, phytol, 9-tricosene, cis-9-hexadecenal, and 17-pentatriacontene. The presence of functional groups of phenyl and aromatic family prominent on the 2nd-stage extract was associated with its high phenolic and flavonoid total, as well as antioxidant activities (Figures 2-4).

3.4. Antioxidant Activity

Primary antioxidant activity of *Sargassum cristaefolium* extracts (Figure 3), the scavenging ability of extracts against free radicals showed that the 2nd-stage extract exhibited the lowest or best IC_{50} value significantly at $p<0.05$ (1439.84±63.02 µg/ml), indicative of superior radical neutralization potential. The final-stage extract (2520.75±135.69 µg/ml) followed closely, with moderate activity observed for the 1st-stage extract (2054.09±68.15 µg/ml). Although the antioxidant activity of the sequenced extracts of *Sargassum cristaefolium* was fainter than both synthetic antioxidants (BHT = 3.99±0.18 µg/ml) and food-standard antioxidants (Ascorbic acid = 1.19±0.05 µg/ml), it was significantly stronger than the antioxidant activity of the distilled water control extract (30512.30±460.20 µg/ml).

Preventive antioxidant activity of *Sargassum cristaefolium* extracts, the chelating capacity for Fe^{2+} ions was presented as IC_{50} values (Figure 4). The chelation of pro-oxidant ferrous ions was significantly (at $p<0.05$) strongest on the 2nd-stage extract (389.73±16.71 µg/ml), demonstrating its preventive antioxidant capability. This was reflected in its superior ability to chelate Fe^{2+} ions compared to the other stages (1st-stage extract = 830.29±37.13 µg/ml and final-stage extract = 1190.48±54.35 µg/ml). The final-stage extract demonstrated the weakest preventive antioxidant capacity. All extract stages had the preventive antioxidant capacity weaker than the standard of ferrous ion chelator, namely EDTA (19.21±0.81 µg/ml). Nevertheless, they are stronger compared to the extract control (5813.95±255.87 µg/ml).

4. Discussion

The present study highlights the effectiveness of MAE in serially extracting bioactive components of *Sargassum cristaefolium* brown seaweed using solvents of different polarities. This experiment successfully extracted diverse bioactive compounds, which demonstrated the significant antioxidant activities, phenolic and flavonoid contents, and rich health benefits, as indicated in Figures 2-5 and Table 2 and 3.

Table 1 presents a comparative analysis of different extraction techniques applied to Sargassum cristaefolium,

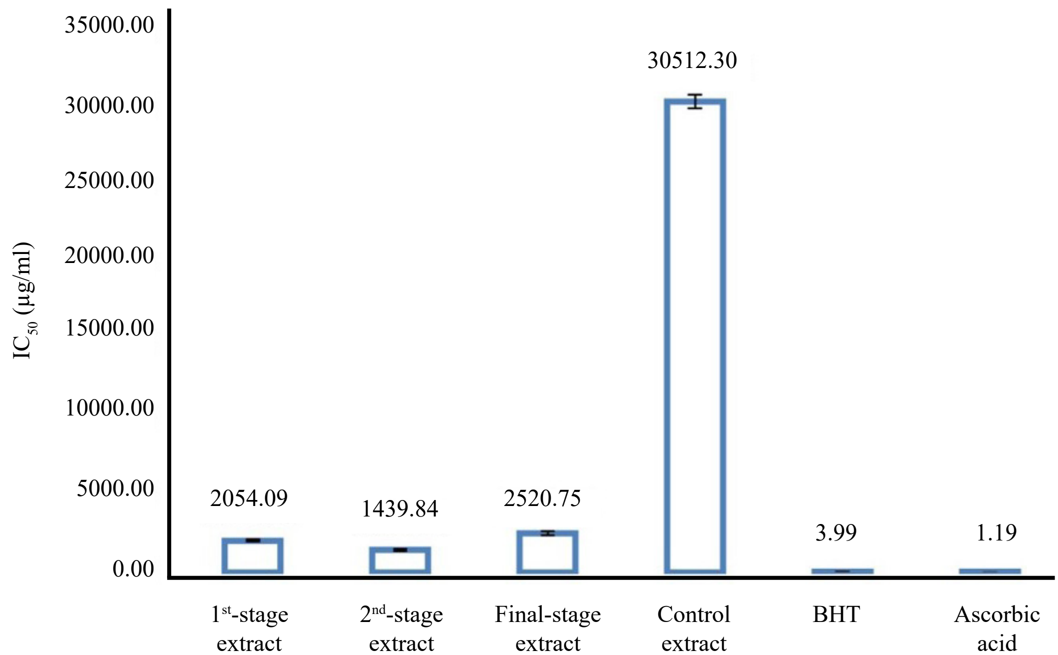


Figure 3. Primary antioxidant activity on free radical scavenging of *Sargassum cristaefolium* brown seaweed extracts

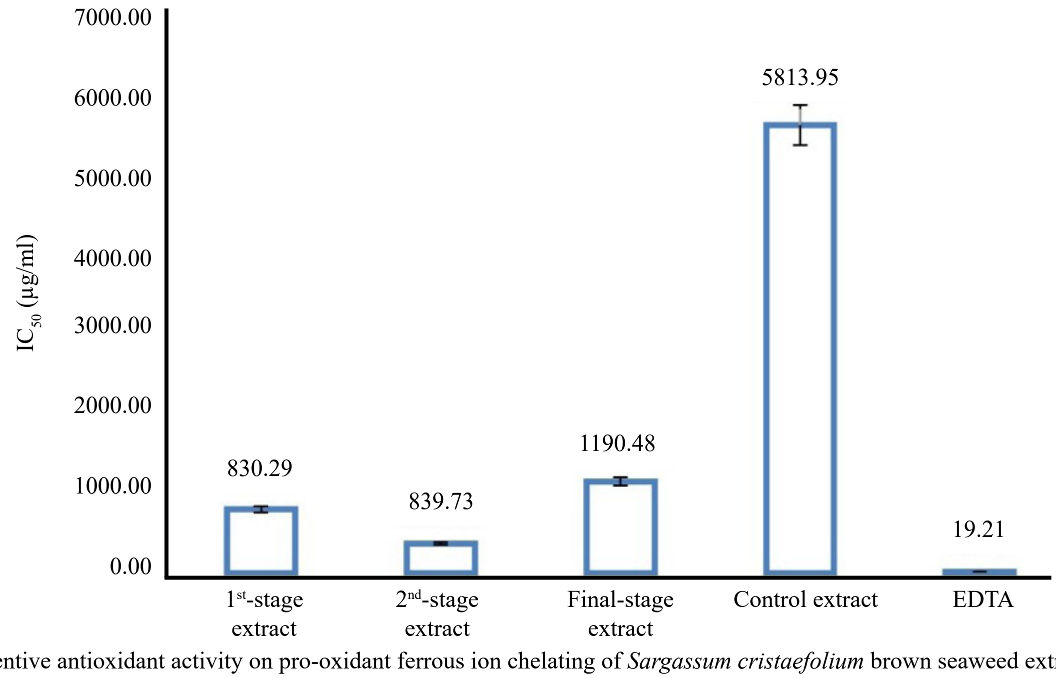


Figure 4. Preventive antioxidant activity on pro-oxidant ferrous ion chelating of *Sargassum cristaefolium* brown seaweed extracts

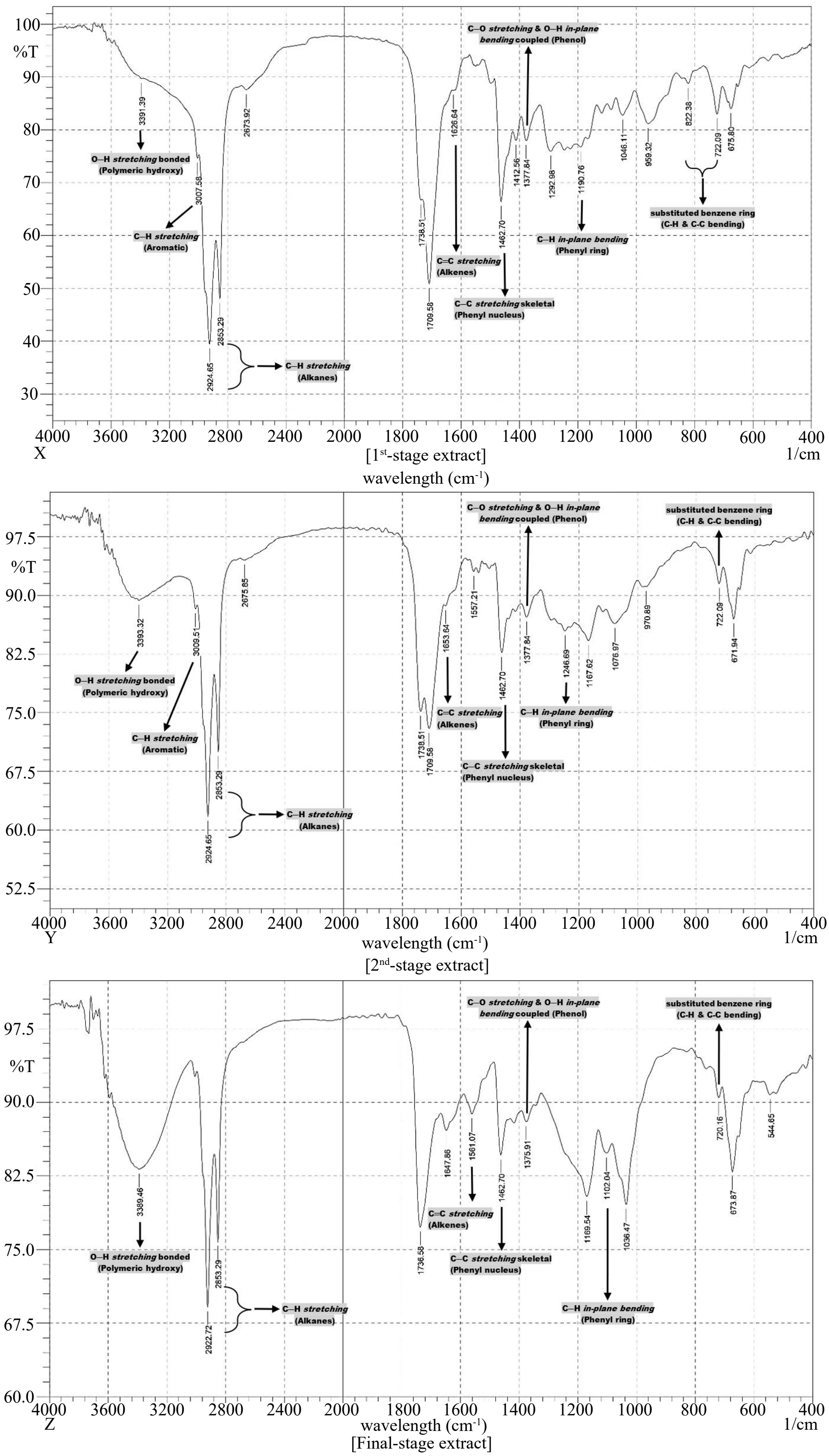


Figure 5. FTIR spectral analysis of *Sargassum cristaefolium* brown seaweed extracts (1st-stage, 2nd-stage, and final-stage extracts)

highlighting the efficiency of MAE in comparison to conventional and advanced methods. The results demonstrate that MAE offers significant advantages in terms of extraction efficiency, yield of bioactive compounds, and antioxidant activity. MAE exhibits higher extraction speeds but may be less selective in targeting specific bioactive compounds without optimized solvents. So, it must pay attention to a used solvent system, enabling selective extraction of phenolic and flavonoid compounds with optimal antioxidant activities. Despite its advantages, MAE also has some limitations. Thermal degradation risks due to microwave heating can negatively affect thermolabile bioactive compounds, potentially reducing the bioactivity of certain sensitive molecules. Moreover, scale-up feasibility remains a challenge, as industrial MAE requires precise optimization of power settings, solvent ratios, and extraction durations to maintain efficiency. Some studies suggested that combining MAE with other techniques, such as enzyme-assisted extraction (EAE) or supercritical fluid extraction (SFE) (Amarante *et al.* 2020; Lourenço-Lopes *et al.* 2023), could further enhance extraction yields and improve selectivity for functional applications.

The serial extraction with non-polar, semi-polar, and polar solvents enabled the selective isolation of distinct classes of bioactive compounds. Notably, the semi-polar solvent (ethyl acetate) yielded the 2nd-stage extract, which had the highest total phenolic and flavonoid contents and superior antioxidant activity. This supports the idea that semi-polar solvents are more effective for solubilizing phenolic and flavonoid compounds, which are known for their antioxidative and therapeutic properties (Deng *et al.* 2015; Payghami *et al.* 2015). In line with Murugan and Iyer (2013) and Zakaria *et al.* (2011) studies, results reported that semi-polar solvents (ethyl acetate) were more significant than other polarity solvents (*n*-hexane, chloroform, methanol, ethanol, acetone, and water) for phenolic and flavonoid extraction of seaweeds. Natural phenolic compounds are known to form more ether, ester, or glycoside compounds than free compounds. The solubility of phenol ester or ether compounds in polar solvents such as water is lower than free phenol compounds (Robinson 1991). Seaweed flavonoid aglycones are less polar than their glycosides. Flavanones are often found as aglycones such as naringenin (Robinson 1991). In addition, flavonoid aglycones, such as the flavanone group, methylated flavones, flavonols, isoflavones, and anthocyanidin aglycones, were less polar. So, it is reported that they are more suitable for extraction using semi-polar solvents such as ethyl acetate, chloroform, dichloromethane, or diethyl ether (Stankovic *et al.* 2011). In contrast, non-polar (*n*-hexane) and polar (methanol) solvents extracted compounds with lower phenolic and flavonoid content, demonstrating that solvent polarity directly influences extraction efficiency. Therefore, the 2nd-stage extract had the highest antioxidant activity as well as significant total phenolic and flavonoid contents, indicating that this research is effective for obtaining antioxidative compounds from *Sargassum cristaefolium* brown seaweed.

The chemical profiles identified through GC-MS and LC-HRMS revealed a wide range of *Sargassum cristaefolium* bioactive compounds with diverse characteristics. This is in good accordance with Majchrzak *et al.* (2018) revelation that all volatile, medium-volatile, low-volatile, and non-volatile organic substances can be completely identified using GC-MS and LC-HRMS consolidations.

GC-MS analysis showed that *Sargassum cristaefolium* extracts contained various significant volatile bioactive compounds. Extraction with semi-polar (2nd-stage extract) and polar (final-stage extract) solvents produced more volatile compounds compared to non-polar (1st-stage extract) solvents. This indicates that volatile compounds in this seaweed are more easily extracted with solvents with medium to high polarity. The biological activities of these compounds, such as antioxidants, anticancer, and anti-inflammatory, indicate their potential for use in pharmaceutical and functional food applications (Esmat *et al.* 2020). Prominently, the 2nd-stage extract was dominant in volatile compounds like neophytadiene and phytol, which possess especially antioxidative, anti-inflammatory, and anticancer properties.

LC-HRMS was used to identify metabolomic bioactive compounds in *Sargassum cristaefolium* extract. LC-HRMS analysis further supported the finding of bioactive compounds, identifying bioactive metabolomic compounds such as tolbutamide, oleoylethanolamide, and R-palmitoyl-(2-methyl) ethanolamide, which exhibit antioxidative, anti-inflammatory, antidiabetic properties, etc. The 1st-stage extract yielded more compounds with hypoglycemic and neuroprotective activities. The 2nd-stage extract focused more on antioxidative, anti-inflammatory, and anticancer bioactivities since this extract contained dominant bioactive compounds such as oleoylethanolamide and R-Palmitoyl-(2-methyl) ethanolamide. The final-stage extract provided a rich compound profile with anti-infective and weight-loss activities. This suggests that Sargassum cristaefolium is a rich source of bioactive compounds with potential for wide applications in health and pharmaceuticals. In addition, the diverse bioactive profiles reinforce the utility of serial extraction for capturing a broad spectrum of health-beneficial compounds.

The FTIR analysis of *Sargassum cristaefolium* extracts (Figure 5) revealed the presence of several functional groups indicative of bioactive compounds. The identification of alkanes, alkenes, aromatic rings, and phenolic structures confirms the presence of polyphenols, flavonoids, and hydrocarbon derivatives. These findings are consistent with previous studies on Sargassum species, where FTIR analysis has been widely used to validate the chemical composition of seaweed extracts and their functional properties (Albratty *et al.* 2021; Moubayed *et al.* 2017). A notable observation from the FTIR spectra was the strong phenolic O–H stretching bands in the 2nd-stage and final-stage extracts, correlating with their high phenolic and flavonoid content, as confirmed by total phenolic and flavonoid assays. The detection of carboxyl (C=O stretching at 1709.58 cm^{-1}) and hydroxyl (O–H stretching at 3393.32 cm^{-1}) groups in the final-stage extract further supports the presence of potent antioxidant compounds. These results align with earlier reports highlighting the structural characteristics of bioactive seaweed-derived polyphenols and their significant antioxidant potential (Končić *et al.* 2011).

Despite the effectiveness of FTIR in functional group identification, some limitations must be acknowledged. FTIR provides only qualitative information, making it necessary to complement this analysis with quantitative techniques such as Nuclear Magnetic Resonance (NMR) or Mass Spectrometry (MS) to precisely determine molecular structures. Additionally, the overlapping absorption bands in complex matrices like seaweed extracts can sometimes lead to ambiguous peak assignments. Alternative methods, such as Two-Dimensional Infrared Spectroscopy (2D-IR) or Raman Spectroscopy, may offer enhanced spectral resolution and minimize such ambiguities (Smith & Dent 2013). Moreover, FTIR spectra interpretation is highly dependent on reference databases and prior knowledge of expected chemical structures, which may introduce subjectivity in peak assignments.

In comparison with other functional group identification techniques applied in marine bioactive compound research, FTIR remains a rapid and cost-effective approach. However, when a more detailed elucidation of chemical structures is required, coupling FTIR with advanced chromatographic techniques such as Gas Chromatography-Mass Spectrometry (GC-MS) or Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) is recommended. Such integrations have been successfully applied in prior studies for a more comprehensive characterization of bioactive compounds in macroalgae (Esmat *et al.* 2020). Future studies should consider employing a combination of spectroscopic and chromatographic techniques to achieve a more detailed chemical profiling of *Sargassum cristaefolium* extracts. The antioxidant activity of the extracts was evaluated using primary (DPPH free radical scavenging) and preventive (ferrous ion chelating) assays. The 2nd-stage extract exhibited the highest activity on DPPH free radical scavenging and pro-oxidative ferrous ion chelation, indicating strong antioxidative potential. These results underscore the role of semi-polar bioactive compounds in neutralizing oxidative stress, a key factor in the prevention of chronic diseases such as cancer and cardiovascular disorders (Končić *et al.* 2011). Although the antioxidant activities were lower than those of antioxidant standards like BHT, EDTA, and ascorbic acid, they were significantly higher than those of the control extract, demonstrating the effectiveness of the organic solvents in extracting functional bioactives of *Sargassum cristaefolium*. These findings underscore the potential of *Sargassum cristaefolium* extracts for functional foods and pharmaceutical applications in the future necessaries. Furthermore, the high phenolic and flavonoid contents, coupled with the observed antioxidant and bioactive profiles in *Sargassum cristaefolium* extracts, provide a scientific basis for potential therapeutics of oxidative stress-related diseases using seaweed-derived materials.

In conclusion, this study successfully demonstrates the potential of *Sargassum cristaefolium* as a rich source of antioxidant and bioactive compounds. It validates the application of MAE with polarity-serially solvent systems for efficient extraction. Each extraction stage produced different bioactive compound profiles, reflecting the success of the gradual method in completely separating compounds based on their polarity, especially the 2nd-stage extraction using a semi-polar solvent, which was the most superior. Microwave technology has proven to be an efficient and effective method of extracting bioactive components from marine natural products. However, optimizing the method of bioactive component recovery with MAE requires a deep study in order for its application in pilot plants or the industrial sector to be feasible.

Conflict of Interest

All author(s) conflicts of interest were absent in any necessary way and at any time. Also, we state that all included data was never previously published in any articles or at any time. The need for data availability could be asked of the corresponding author (abd.rohim310592@gmail.com; rohim@itsnupasuruan.ac.id).

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References

Abou-Elnour, F.S., El-Habashy, S.E., Essawy, M.M., Abdallah, O.Y., 2024. Codelivery of ivermectin and methyl dihydrojasmonate in nanostructured lipid carrier for synergistic antileukemia therapy. *Int J Pharm.* 656, 124086. https://doi.org/10.1016/J.IJPHARM.2024.124086

Albratty, M., Bajawi, A.A.M., Marei, T.M.H., Alam, M.S., Alhazmi, H.A., Najmi, A., Rehman, Z.U., Moni, S.S., 2021. Spectral analysis and antibacterial potential of bioactive principles of *Sargassum crassifolium* J. *Agardh from Red sea of Jazan origin. Saudi Journal of Biological Sciences*. 28, 5745-5753. https://doi.org/10.1016/j.sjbs.2021.06.017

Aïssaoui, H., Mencherini, T., Esposito, T., De Tommasi, N., Gazzerro, P., Benayache, S., Benayache, F., Mekkiou, R., 2019. *Heliotropium bacciferum* Forssk. (Boraginaceae) extracts: chemical constituents, antioxidant activity and cytotoxic effect in human cancer cell lines. *Nat Prod Res.* 33, 1813–1818. https://doi.org/10.1080/14786419.2018.1437433

AL-Eitan, L., Kharmah, H.A., 2024. Effect of EMB-FUBINACA on brain endothelial cell angiogenesis: expression analysis of angiogenic markers. *Naunyn Schmiedebergs Arch Pharmacol.* 2024, 1–12. https://doi.org/10.1007/s00210-024-03322-1

Alves, J., Gaspar, H., Silva, J., Alves, C., Martins, A., Teodoro, F., Susano, P., Pinteus, S., Pedrosa, R., 2021. Unravelling the anti-inflammatory and antioxidant potential of the marine sponge cliona celata from the portuguese coastline. *Mar Drugs* 19, 632. https://doi.org/10.3390/MD19110632/S1

Amarante, S.J., Catarino, M.D., Marçal, C., Silva, A.M.S., Ferreira, R., Cardoso, S.M., 2020. Microwave-assisted extraction of phlorotannins from *Fucus vesiculosus. Marine Drugs.* 18, 559. https://doi.org/10.3390/MD18110559

Ameamsri, U., Chaveerach, A., Sudmoon, R., Tanee, T., Peigneur, S., Tytgat, J., 2020. Oleamide in *Ipomoea* and *Dillenia* species and inflammatory activity investigated through ion channel inhibition. *Curr Pharm Biotechnol.* 22, 254–261. https://doi.org/10.2174/1389201021666200607185250

Arumugam, M.K., Paal, M.C., Donohue, T.M., Ganesan, M., Osna, N.A., Kharbanda, K.K., 2021. Beneficial effects of betaine: a comprehensive review. *Biology.* 10, 456. https://doi.org/10.3390/BIOLOGY10060456

Aswani, R., Das, S., Theresa, M., Sebastian, K.S., Mathew, J., Radhakrishnan, E.K., 2023. 9-Tricosene containing blend of volatiles produced by *Serratia* sp. NhPB1 isolated from the pitcher plant provide plant protection against *Pythium aphanidermatum. Appl Biochem Biotechnol.* 195, 6098–6112. https://doi.org/10.1007/S12010-023-04352-W/TABLES/1

Bahrun, Okino, T., Rasyid, H., Soekamto, N.H., 2023. Biological evaluation and molecular docking of Indonesian *Gracilaria salicornia* as antioxidant agents. *Journal of Research in Pharmacy* 27, 207. https://doi.org/10.29228/JRP.304

Bhardwaj, M., Sali, V.K., Mani, S., Vasanthi, H.R., 2020. Neophytadiene from *Turbinaria ornata* suppresses LPS-induced inflammatory response in RAW 264.7 macrophages and sprague dawley rats. *Inflammation.* 43, 937–950. https://doi.org/10.1007/S10753-020-01179-Z/FIGURES/5

Bialer, M., 1991. Clinical pharmacology of valpromide. *Clin Pharmacokinet.* 20, 114–122. https://doi.org/10.2165/00003088-199120020-00003/METRICS

Blusztajn, J.K., Slack, B.E., Mellott, T.J., 2017. Neuroprotective actions of dietary choline. *Nutrients.* 9, 815. https://doi.org/10.3390/NU9080815

Bryology, A., Dergisi, A.B., Turu, D., Deniz Bozkurt, S., Yaman, C., Gül, G., Benek, A., Canli, K., 2024. Determination of biochemical content and antioxidant activity of *Calliergonella cuspidata* (Hedw.) loeske. *Anatolian Bryology.* 10, 25-33. https://doi.org/10.26672/ANATOLIANBRYOLOGY.1434173

Chakraborty, M., Ahmed, M.G., Bhattacharjee, A., 2017. The potential for interaction of tolbutamide with pomegranate juice against diabetic induced complications in rats. *Integr Med Res.* 6, 354-360. https://doi.org/10.1016/J.IMR.2017.07.006

Chakraborty, S., Rakshit, J., Bandyopadhyay, J., Basu, S., 2018. Multi-functional neuroprotective activity of neohesperidin dihydrochalcone: a novel scaffold for Alzheimer's disease therapeutics identified via drug repurposing screening. *New Journal of Chemistry.* 42, 11755–11769. https://doi.org/10.1039/C8NJ00853A

Chan, Y., Singh, S.K., Gulati, M., Wadhwa, S., Prasher, P., Kumar, D., Kumar, A.P., Gupta, G., Kuppusamy, G., Haghi, M., George Oliver, B.G., Adams, J., Chellappan, D.K., Dua, K., 2022. Advances and applications of monoolein as a novel nanomaterial in mitigating chronic lung diseases. *J Drug Deliv Sci Technol.* 74, 103541. https://doi.org/10.1016/J.JDDST.2022.103541

Charles, A.K., Darbre, P.D., 2009. Oestrogenic activity of benzyl salicylate, benzyl benzoate and butylphenylmethylpropional (Lilial) in MCF7 human breast cancer cells *in vitro. Journal of Applied Toxicology.* 29, 422-434. https://doi.org/10.1002/JAT.1429

Cheng, M.C., Chang, W.H., Chen, C.W., Li, W.W., Tseng, C.Y., Song, T.Y., 2015. Antioxidant properties of essential oil extracted from *Pinus morrisonicola* Hay needles by supercritical fluid and identification of possible active compounds by GC/MS. *Molecules* 20, 19051-19065. https://doi.org/10.3390/MOLECULES201019051

D'aloia, A., Arrigoni, F., Tisi, R., Palmioli, A., Ceriani, M., Artusa, V., Airoldi, C., Zampella, G., Costa, B., Cipolla, L., 2020. Synthesis, molecular modeling and biological evaluation of metabolically stable analogues of the endogenous fatty acid amide palmitoylethanolamide. *International Journal of Molecular Sciences.* 21, 9074. https://doi.org/10.3390/IJMS21239074

Deng, B., Cao, Y., Fang, S., Shang, X., Yang, W., Qian, C., 2015. Variation and stability of growth and leaf flavonoid content in *Cyclocarya paliurus* across environments. *Ind Crops Prod.* 76, 386–393. https://doi.org/10.1016/J.INDCROP.2015.07.011

Devi, T.S., Vijay, K., Vidhyavathi, R.M., Kumar, P., Govarthanan, M., Kavitha, T., 2021. Antifungal activity and molecular docking of phenol, 2,4-bis(1,1-dimethylethyl) produced by plant growth-promoting actinobacterium *Kutzneria* sp. strain TSII from mangrove sediments. *Arch Microbiol.* 203, 4051–4064. https://doi.org/10.1007/S00203-021-02397-1/FIGURES/5

Erukainure, O.L., Mesaik, A.M., Muhammad, A., Chukwuma, C.I., Manhas, N., Singh, P., Aremu, O.S., Islam, M.S., 2016. Flowers of *Clerodendrum volubile* exacerbate immunomodulation by suppressing phagocytic oxidative burst and modulation of COX-2 activity. *Biomedicine & Pharmacotherapy.* 83, 1478-1484. https://doi.org/10.1016/J.BIOPHA.2016.09.002

Esmaeili, A., Jafarzadeh, F., 2016. Nanocatalyst transformation and biological activities of lilial. *Particulate Science and Technology.* 34, 33–38. https://doi.org/10.1080/02726351.2015.1039099

Esmat, A.U., Mittapally, S., Begum, S., 2020. GC-MS analysis of bioactive compounds and phytochemical evaluation of the ethanolic extract of *Gomphrena globosa* L. flowers. *Journal of Drug Delivery and Therapeutics.* 10, 53–58. https://doi.org/10.22270/JDDT.V10I2.3914

G, D.K., M, K., R, R., 2018. GC-MS analysis of bioactive compounds from ethanolic leaves extract of *Eichhornia crassipes* (Mart) solms. and their pharmacological activities. *The Pharma Innovation Journal.* 7, 459–462.

Gahtori, R., Tripathi, A.H., Chand, G., Pande, A., Joshi, P., Rai, R.C., Upadhyay, S.K., 2024. Phytochemical screening of *Nyctanthes arbor-tristis* plant extracts and their antioxidant and antibacterial activity analysis. *Appl Biochem Biotechnol.* 196, 436–456. https://doi.org/10.1007/S12010-023-04552-4/FIGURES/5

Ghaffari, S., Roshanravan, N., Tutunchi, H., Ostadrahimi, A., Pouraghaei, M., Kafil, B., 2020. Oleoylethanolamide, a bioactive lipid amide, as a promising treatment strategy for coronavirus/COVID-19. *Arch Med Res.* 51, 464–467. https://doi.org/10.1016/J.ARCMED.2020.04.006

Golam Mostofa, M., Ali Reza, A.S., Khan, Z., Khurshid Alam, A., Golam Sadik, M., 2021. The apoptosis-inducing antiproliferative activity and quantitative phytochemical profiling of polyphenol-rich part of *Leea aequata* L. leaves. *Heliyon.* 10, e23400. https://doi.org/10.21203/RS.3.RS-830741/V1

Gołebiowski, M., Cerkowniak, M., Urbanek, A., Dawgul, M., Kamysz, W., Boguś, M.I., Stepnowski, P., 2015. Identification and antifungal activity of novel organic compounds found in cuticular and internal lipids of medically important flies. *Microbiol Res.* 170, 213–222. https://doi.org/10.1016/J.MICRES.2014.06.004

Gonzalez-Rivera, M.L., Barragan-Galvez, J.C., Gasca-Martínez, D., Hidalgo-Figueroa, S., Narayanankutty, A., Famurewa, A.C., Gonzalez-Rivera, M.L., Carlos Barragan-Galvez, J., Gasca-Martínez, Deisy, Hidalgo-Figueroa, Sergio, Isiordia-Espinoza, M., Alonso-Castro, A.J., 2023. *In vivo* neuropharmacological effects of neophytadiene. *Molecules.* 28, 3457. https://doi.org/10.3390/MOLECULES28083457

Grossmann, M.E., Mizuno, N.K., Dammen, M.L., Schuster, T., Ray, A., Cleary, M.P., 2009. Eleostearic acid inhibits breast cancer proliferation by means of an oxidation-dependent mechanism. *Cancer Prevention Research.* 2, 879–886. https://doi.org/10.1158/1940-6207.CAPR-09-0088/339179/P/ELEOSTEARIC-ACID-INHIBITS-BREAST-CANCER

Gupta, K., Taj, T., Thansiya, B., V Kamath, J., 2019. Pre-clinical evaluation of hepatoprotective activity of phytol in wistar albino rats. *IP International Journal of Comprehensive and Advanced Pharmacology.* 4, 17–20. https://doi.org/10.18231/J.IJCAAP.2019.004

Gupta, P., Banerjee, A., Castillo, A., Bandopadhyay, R., 2021. Novel phenolic compound from Southern Ocean microalgae *Chlorella* sp. PR-1 and its antibacterial activity. *Gayana Bot.* 78, 29–37.

Heng, Y.W., Ban, J.J., Khoo, K.S., Sit, N.W., 2020. Biological activities and phytochemical content of the rhizome hairs of *Cibotium barometz* (Cibotiaceae). *Ind Crops Prod.* 153, 112612. https://doi.org/10.1016/J.INDCROP.2020.112612

Hoda, S., Gupta, L., Agarwal, H., Raj, G., Vermani, M., Vijayaraghavan, P., 2019. Inhibition of aspergillus fumigatus biofilm and cytotoxicity study of natural compound Cis-9-Hexadecenal. *J Pure Appl Microbiol.* 13, 1207–1216. https://doi.org/10.22207/JPAM.13.2.61

Hoseini, S.M., Gharavi, B., Taheri Mirghaed, A., Hoseinifar, S.H., Van Doan, H., 2021. Effects of dietary phytol supplementation on growth performance, immunological parameters, antioxidant and stress responses to ammonia exposure in common carp, *Cyprinus carpio* (Linnaeus, 1758). *Aquaculture.* 545, 737151. https://doi.org/10.1016/J.AQUACULTURE.2021.737151

Hu, L., Li, L., Xu, Demei, Xia, X., Pi, R., Xu, Duo, Wang, W., Du, H., Song, E., Song, Y., 2014. Protective effects of neohesperidin dihydrochalcone against carbon tetrachloride-induced oxidative damage *in vivo* and *in vitro*. *Chem Biol Interact.* 213, 51–59. https://doi.org/10.1016/J.CBI.2014.02.003

Huang, X., Xu, N., Liu, Z., Li, H., Lu, H., Li, J., 2024. Chemical composition of *Achillea millefolium* L. and their anti-inflammatory activity. *Chem Biodivers.* 21, e202400946. https://doi.org/10.1002/CBDV.202400946

Imran, M., Titilayo, B., Adil, M., Liyan-Zhang, Mehmood, Q., Mustafa, S.H., Shen, Q., 2024. Ascorbyl palmitate: a comprehensive review on its characteristics, synthesis, encapsulation and applications. *Process Biochemistry.* 142, 68–80. https://doi.org/10.1016/J.PROCBIO.2024.04.015

Islam, M.T., Ali, E.S., Uddin, S.J., Shaw, S., Islam, M.A., Ahmed, M.I., Chandra Shill, M., Karmakar, U.K., Yarla, N.S., Khan, I.N., Billah, M.M., Pieczynska, M.D., Zengin, G., Malainer, C., Nicoletti, F., Gulei, D., Berindan-Neagoe, I., Apostolov, A., Banach, M., Yeung, A.W.K., El-Demerdash, A., Xiao, J., Dey, P., Yele, S., Jóźwik, A., Strzałkowska, N., Marchewka, J., Rengasamy, K.R.R., Horbańczuk, J., Kamal, M.A., Mubarak, M.S., Mishra, S.K., Shilpi, J.A., Atanasov, A.G., 2018. Phytol: a review of biomedical activities. *Food and Chemical Toxicology.* 121, 82–94. https://doi.org/10.1016/J.FCT.2018.08.032

Islas-Garduño, A.L., Romero-Cerecero, O., Jiménez-Aparicio, A.R., Tortoriello, J., Montiel-Ruiz, R.M., González-Cortazar, M., Zamilpa, A., 2023. Pharmacological and chemical analysis of *Bauhinia divaricata* L. using an *in vitro* antiadipogenic model. *Plants.* 12, 3799. https://doi.org/10.3390/PLANTS12223799

Karthikeyan, S.C., Velmurugan, S., Donio, M.B.S., Michaelbabu, M., Citarasu, T., 2014. Studies on the antimicrobial potential and structural characterization of fatty acids extracted from Sydney rock oyster *Saccostrea glomerata. Ann Clin Microbiol Antimicrob.* 13, 1–11. https://doi.org/10.1186/S12941-014-0057-X/TABLES/2

Kim, C.R., Choi, S.J., Kim, J.K., Park, C.K., Gim, M.C., Kim, Y.J., Park, G.G., Shin, D.H., 2017. 2,4-Bis(1,1-dimethylethyl) phenol from *Cinnamomum loureirii* improves cognitive deficit, cholinergic dysfunction, and oxidative damage in TMT-treated mice. *Biol Pharm Bull.* 40, 932–935. https://doi.org/10.1248/BPB.B16-00997

Kim, C.R., Kim, H.S., Choi, S.J., Kim, J.K., Gim, M.C., Kim, Y.J., Shin, D.H., 2018. Erucamide from radish leaves has an inhibitory effect against acetylcholinesterase and prevents memory deficit induced by trimethyltin. *Journal of Medicinal Food.* 21, 769–776. https://doi.org/10.1089/JMF.2017.4117

Končić, M.Z., Barbarić, M., Perković, I., Zorc, B., 2011. Antiradical, chelating and antioxidant activities of hydroxamic acids and hydroxyureas. *Molecules.* 16, 6232–6242. https://doi.org/10.3390/MOLECULES16086232

Kuroda, Y., Yamagata, H., Nemoto, M., Inagaki, K., Tamura, T., Maeda, K., 2019. Antiviral effect of sinefungin on *in vitro* growth of feline herpesvirus type 1. *The Journal of Antibiotics* 72, 981–985. https://doi.org/10.1038/s41429-019-0234-4

Lann, K. Le, Ferret, C., Vanmee, E., Spagnol, C., Lhuillery, M., Payri, C., Stiger-Pouvreau, V., 2012. Total phenolic, size-fractionated phenolics and fucoxanthin content of tropical sargassaceae (Fucales, Phaeophyceae) from the South Pacific Ocean: spatial and specific variability. *Phycological Res.* 60, 37–50. https://doi.org/10.1111/J.1440-1835.2011.00634.X

Lee, Y.S., Chang, Z.Q., Oh, B.C., Park, S.C., Shin, S.R., Kim, N.W., 2007. Antioxidant activity, anti-inflammatory activity, and whitening effects of extracts of *Elaeagnus multiflora* Thunb. *Journal of Medicinal Food.* 10, 126–133. https://doi.org/10.1089/JMF.2006.145

Li, M.M., Jiang, Z. er, Song, L.Y., Quan, Z.S., Yu, H.L., 2017. Antidepressant and anxiolytic-like behavioral effects of erucamide, a bioactive fatty acid amide, involving the hypothalamus–pituitary–adrenal axis in mice. *Neurosci Lett.* 640, 6–12. https://doi.org/10.1016/J.NEULET.2016.12.072

Lima, M.L., Romanelli, M.M., Borborema, S.E.T., Johns, D.M., Migotto, A.E., Lago, J.H.G., Tempone, A.G., 2019. Antitrypanosomal activity of isololiolide isolated from the marine hydroid *Macrorhynchia philippina* (Cnidaria, Hydrozoa). *Bioorg Chem.* 89, 103002. https://doi.org/10.1016/J.BIOORG.2019.103002

Lourenço-Lopes, C., Carreira-Casais, A., Carperna, M., Barral-Martinez, M., Chamorro, F., Jiménez-López, C., Cassani, L., Simal-Gandara, J., Prieto, M.A., 2023. Emerging technologies to extract fucoxanthin from *Undaria pinnatifida*: microwave vs. ultrasound assisted extractions. *Marine Drugs.* 21, 282. https://doi.org/10.3390/MD21050282

Majchrzak, T., Wojnowski, W., Lubinska-Szczygeł, M., Różańska, A., Namieśnik, J., Dymerski, T., 2018. PTR-MS and GC-MS as complementary techniques for analysis of volatiles: a tutorial review. *Anal Chim Acta.* 1035, 1–13. https://doi.org/10.1016/J.ACA.2018.06.056

Makajanma, M.M., Taufik, I., Faizal, A., 2020. Antioxidant and antibacterial activity of extract from two species of mosses: *Leucobryum aduncum* and *Campylopus schmidii. Biodiversitas.* 21, 2751–2758. https://doi.org/10.13057/BIODIV/D210651

Malekhayati, H., Bargahi, A., Khorami, S., Khataminejad, M., Fouladvand, M., 2024. Anti-*Trichomonas vaginalis* activity of marine ascidians (Tunicates; Ascidiacea) from the Bushehr Province, Iran. *Turkiye Parazitoloji Dergisi.* 48, 21–26. https://doi.org/10.4274/TPD.GALENOS.2023.96658

Mou, Y., Meng, J., Fu, X., Wang, X., Tian, J., Wang, M., Peng, Y., Zhou, L., 2013. Antimicrobial and antioxidant activities and effect of 1-Hexadecene addition on Palmarumycin C2 and C3 yields in liquid culture of endophytic fungus *Berkleasmium* sp. Dzf12. *Molecules.* 18, 15587-15599. https://doi.org/10.3390/MOLECULES181215587

Moubayed, N.M.S., Al Houri, H.J., Al Khulaifi, M.M., Al Farraj, D.A., 2017. Antimicrobial, antioxidant properties and chemical composition of seaweeds collected from Saudi Arabia (Red Sea and Arabian Gulf). *Saudi J Biol Sci.* 24, 162–169. https://doi.org/10.1016/J.SJBS.2016.05.018

Murugan, K., Iyer, V.V., 2013. Differential growth inhibition of cancer cell lines and antioxidant activity of extracts of red, brown, and green marine algae. *In Vitro Cell Dev Biol Anim.* 49, 324–334. https://doi.org/10.1007/S11626-013-9603-7/FIGURES/4

Nishimura, Y., Nomiyama, K., Okamoto, S., Igarashi, M., Sato, Y., Okamoto, H., Kamezaki, A., Itadani, M., Kuribayashi, F., Yamauchi, A., 2024. Anti-fatigue activity of methyl dihydrojasmonate and linalool in a rat model evaluated by a novel index for neuro-immune and oxidative stress interactions. *Scientific Reports.* 14, 1–10. https://doi.org/10.1038/s41598-024-60266-5

Nmeazi, O.F., Beverly, O.M., Awolayeofori, D., Nornubari, N.J., Nkomadu, V.D., Odinga, T.B., 2024. Evaluation of the bioactive composition and *in vitro* neuroprotective potential of the combined *Curcuma longa* and *Rosmarinus officinalis* ethanol extract. *Trends in Medical Research.* 19, 136–150. https://doi.org/10.3923/TMR.2024.136.150

Nolan, L.L., 1987. Molecular target of the antileishmanial action of sinefungin. *Antimicrob Agents Chemother.* 31, 1542–1548. https://doi.org/10.1128/AAC.31.10.1542

Okpala, E.O., Onocha, P.A., Ali, M.S., 2022. Antioxidant activity of phytol dominated stem bark and leaf essential oils of *Celtis zenkeri* Engl. *Trends in Phytochemical Research.* 137 2, 137. https://doi.org/10.30495/TPR.2022.1952985.1246

Olasehinde, T.A., Olaniran, A.O., Okoh, A.I., 2021. Cholinesterase inhibitory activity, antioxidant properties, and phytochemical composition of *Chlorococcum* sp. extracts. *J Food Biochem.* 45, e13395. https://doi.org/10.1111/JFBC.13395

Olejnik, A., Sliwowska, A., Nowak, I., 2018. Jasmonic acid, methyl jasmonate and methyl dihydrojasmonate as active compounds of topical formulations. *Colloids Surf A Physicochem Eng Asp.* 558, 558–569. https://doi.org/10.1016/J.COLSURFA.2018.09.026

Onanuga, A.O., Okpala, E.O., 2022. Chemical compositions and antioxidant activity of volatile oils from *Morinda citrifolia* and *Beta vulgaris* leaves from Nigeria. *Biology, Medicine, & Natural Product Chemistry.* 11, 161–167. https://doi.org/10.14421/BIOMEDICH.2022.112.161-167

Park, H., Kim, B., Kang, Y., Kim, W., 2024. Study on chemical composition and biological activity of *Psidium guajava* leaf extracts. *Current Issues in Molecular Biology.* 46, 2133–2143. https://doi.org/10.3390/CIMB46030137

Payghami, N., Jamili, S., Rustaiyan, A., Saeidnia, S., Nikan, M., Gohari, A.R., 2015. Alpha amylase inhibitory activity and sterol composition of the marine algae, *Sargassum glaucescens. Pharmacognosy Res.* 7, 314–321. https://doi.org/10.4103/0974-8490.167893

Pejin, Boris, Kojic, V., Bogdanovic, G., 2014a. An insight into the cytotoxic activity of phytol at *in vitro* conditions. *Nat Prod Res.* 28, 2053–2056. https://doi.org/10.1080/14786419.2014.921686

Pejin, B., Savic, A., Sokovic, M., Glamoclija, J., Ciric, A., Nikolic, M., Radotic, K., Mojovic, M., 2014b. Further *in vitro* evaluation of antiradical and antimicrobial activities of phytol. *Nat Prod Res.* 28, 372–376. https://doi.org/10.1080/14786419.2013.869692

Prasedya, E.S., Frediansyah, A., Martyasari, N.W.R., Ilhami, B.K., Abidin, A.S., Padmi, H., Fahrurrozi, Juanssilfero, A.B., Widyastuti, S., Sunarwidhi, A.L., 2021a. Effect of particle size on phytochemical composition and antioxidant properties of *Sargassum cristaefolium* ethanol extract. *Sci Rep.* 11, 17876. https://doi.org/10.1038/s41598-021-95769-y

Prasedya, E.S., Martyasari, N.W.R., Abidin, A.S., Ilhami, B.T.K., Padmi, H., Widyastuti, S., Sunarwidhi, A.L., Sunarpi, H., 2021b. Antioxidant activity of brown macroalgae *Sargassum* ethanol extract from Lombok coast, Indonesia. *IOP Conf Ser Earth Environ Sci.* 712, 012038. https://doi.org/10.1088/1755-1315/712/1/012038

Quarterman, J.C., Naguib, Y.W., Chakka, J.L., Seol, D., Martin, J.A., Salem, A.K., 2022. HPLC-UV method validation for amobarbital and pharmaceutical stability evaluation when dispersed in a hyaluronic acid hydrogel: a new concept for post-traumatic osteoarthritis prevention. *J Pharm Sci.* 111, 1379–1390. https://doi.org/10.1016/J.XPHS.2021.09.025

Rahamtulla, M., Mallikarjuna, K., Khasim, S.M., 2023. GC-MS analysis and therapeutic importance of leaf extracts of *Dendrobium aphyllum* (Roxb.) C.E.C. fischer: an *in vitro* study. *South African Journal of Botany.* 153, 62–76. https://doi.org/10.1016/J.SAJB.2022.12.011

Ratnayake, R., Liu, Y., Paul, V.J., Luesch, H., 2013. Cultivated sea lettuce is a multiorgan protector from oxidative and inflammatory stress by enhancing the endogenous antioxidant defense system. *Cancer Prevention Research.* 6, 989–999. https://doi.org/10.1158/1940-6207.CAPR-13-0014

Reichardt, C., Welton, T., 2010. *Solvents and Solvent Effects in Organic Chemistry*, fourth ed. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. https://doi.org/10.1002/9783527632220

Ren, J., Wang, J., Karthikeyan, S., Liu, H., Cai, J., 2019. Natural anti-phytopathogenic fungi compound phenol, 2, 4-bis (1, 1-dimethylethyl) from *Pseudomonas fluorescens* TL-1. *IJBB*. 56, 162–168.

Rengasamy, K.R.R., Sadeer, N.B., Zengin, G., Mahomoodally, M.F., Cziáky, Z., Jekő, J., Diuzheva, A., Abdallah, H.H., Kim, D.H., 2019. Biopharmaceutical potential, chemical profile and *in silico* study of the seagrass-*Syringodium isoetifolium* (Asch.) Dandy. *South African Journal of Botany.* 127, 167–175. https://doi.org/10.1016/J.SAJB.2019.08.043.

Robinson, T., 1991. *The Organic Constituens of Higher Plants*, sixth ed. Cordus Press, USA.

Rongpan, S., Trongwongsa, T., Thanomsub, B.W., Jariyapongskul, A., 2558. Effect of monoolein on inhibition of tumor growth in cervical cancer xenografts in nude mice. *Journal of Medicine and Health Science.* 22, 41–52.

Saha, S.S., Ghosh, M., 2009. Comparative study of antioxidant activity of α-eleostearic acid and punicic acid against oxidative stress generated by sodium arsenite. *Food and Chemical Toxicology.* 47, 2551–2556. https://doi.org/10.1016/J.FCT.2009.07.012

Saraswati, Giantina, G., Giriwono, P.E., Faridah, D.N., Iskandriati, D., Andarwulan, N., 2020. Water and lipid-soluble component profile of *Sargassum cristaefolium* from different coastal areas in Indonesia with potential for developing functional ingredient. *J Oleo Sci.* 69, 1517–1528. https://doi.org/10.5650/JOS.ESS20079

Sati, P., Sharma, E., Dhyani, P., Attri, D.C., Rana, R., Kiyekbayeva, L., Büsselberg, D., Samuel, S.M., Sharifi-Rad, J., 2024. Paclitaxel and its semi-synthetic derivatives: comprehensive insights into chemical structure, mechanisms of action, and anticancer properties. *European Journal of Medical Research.* 29, 1-26. https://doi.org/10.1186/S40001-024-01657-2

Sharaf, M.H., 2020. Evaluation of the antivirulence activity of ethyl acetate extract of *Deverra tortuosa* (Desf) against *Candida albicans. Egyptian Pharmaceutical Journal.* 19, 188–196. https://doi.org/10.4103/EPJ.EPJ_10_20

Silva, D., Bambaranda, B.V.A.S.M., Mudannayake, D.C., 2022. Bioactive, microbiological, and sensory properties of *Sargassum cristaefolium* and *Sargassum crassifolium* herbal tea. *Advances in Technology.* 2, 38–49. https://doi.org/10.31357/AIT.V2I1.5456

Silva, R.O., Sousa, F.B.M., Damasceno, S.R.B., Carvalho, N.S., Silva, V.G., Oliveira, F.R.M.A., Sousa, D.P., Aragão, K.S., Barbosa, A.L.R., Freitas, R.M., Medeiros, J.V.R., 2014. Phytol, a diterpene alcohol, inhibits the inflammatory response by reducing cytokine production and oxidative stress. *Fundam Clin Pharmacol.* 28, 455–464. https://doi.org/10.1111/FCP.12049

Smith, E., Dent, G., 2019. *Modern Raman Spectroscopy: a Practical Approach*, second ed. John Wiley & Sons Ltd., United Kingdom.

Song, W.Y., Peng, S.P., Shao, C.Y., Shao, H.B., Yang, H.C., 2014. Ethylene glycol tetra-acetic acid and salicylic acid improve antioxidative ability of maize seedling leaves under heavy-metal and polyethylene glycol 6000-simulated drought stress. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology* 148, 96–108. https://doi.org/10.1080/11263504.2013.878408

Sova, M., 2012. Antioxidant and antimicrobial activities of cinnamic acid derivatives. *Mini-Reviews in Medicinal Chemistry.* 12, 749–767. https://doi.org/10.2174/138955712801264792

Stankovic, M.S., Niciforovic, N., Topuzovic, M., Solujic, S., 2011. Total phenolic content, flavonoid concentrations and antioxidant activity, of the whole plant and plant parts extracts from *Teucrium montanum* L. var. Montanum, F. *Supinum* (L.) Reichenb. *Biotechnology & Biotechnological Equipment.* 25, 2222–2227. https://doi.org/10.5504/BBEQ.2011.0020

Sunarwidhi, A.L., Hernawan, A., Frediansyah, A., Widyastuti, S., Martyasari, N.W.R., Abidin, A.S., Padmi, H., Handayani, E., Utami, N.W.P., Maulana, F.A., Ichfa, M.S.M., Prasedya, E.S., 2022. Multivariate analysis revealed ultrasonic-assisted extraction improves anti-melanoma activity of non-flavonoid compounds in Indonesian brown algae ethanol extract. *Molecules.* 27, 7509. https://doi.org/10.3390/MOLECULES27217509/S1

Sunarwidhi, A.L., Rahmaniar, W., Prasedya, E.S., Padmi, H., Widyastuti, S., Pangestu, K.W.J., Ilhami, B.T.K., Handayani, E., Utami, N.W.P., Maulana, F.A., Ichfa, M.S.M., Hernawan, A., 2023. *In vitro* antioxidant, *in vivo* anti-hyperglycemic, and untargeted metabolomics-aided-*in silico* screening of macroalgae lipophilic extracts for anti-diabetes mellitus and anti-COVID-19 potential metabolites. *Metabolites.* 13, 1177. https://doi.org/10.3390/metabo13121177

Susilo, B., Lestari W.H., M., Rohim, Abd., 2020. Impact of using low-cost packaging material of commercial herbal oil on its antibacterial compounds. *All Life*. 13, 516–523. https://doi.org/10.1080/26895293.2020.1817800

Susilo, B., Oktavianty, O., Rahayu, F., Handayani, M.L.W., Rohim, A., 2023a. Potential transformation of seagrass (*Syringodium isoetifolium*) into a bioactive food ingredient using different extraction techniques. *F1000Research.* 12, 1078. https://doi.org/10.12688/f1000research.128718.1

Susilo, B., Rohim, A., Filayati, M.A.J., 2022. Vacuum drying as a natural preservation method of post-harvest lemon might accelerate drying duration and produce the high-quality of dried lemon slices. *Food Science and Technology.* 42, 1-6. https://doi.org/10.1590/FST.58722

Susilo, B., Rohim, A., Wirawan, Handayani, M.L.W., Azis, M.N., Haryono, M.E., Junaidy S, A., Nahak, A., 2024. Extraction of bioactive phytochemicals on Berlin banana flower (*Musa acuminate* AA.) using continuous system-designed ultrasound machine. *Cogent Food Agric.* 10, 2410477. https://doi.org/10.1080/23311932.2024.2410477

Susilo, B., Setyawan, H.Y., Prianti, D.D., Handayani, M.L.W., Rohim, A., 2023b. Extraction of bioactive components on Indonesian seagrass (*Syringodium isoetifolium*) using green emerging technology. *Food Science and Technology.* 43, e086722. https://doi.org/10.1590/FST.086722

T, B., Vigasini, N., 2021. Antioxidant and antidiabetic activities of ethanolic extract of *Hibiscus sabdariffa* calyx and *Stevia rebaudiana* Leaf. *Asian J Biol Life Sci.* 10, 217–224. https://doi.org/10.5530/AJBLS.2021.10.31

Thabuis, C., Tissot-Favre, D., Bezelgues, J.B., Martin, J.C., Cruz-Hernandez, C., Dionisi, F., Destaillats, F., 2008. Biological functions and metabolism of oleoylethanolamide. *Lipids.* 43, 887–894. https://doi.org/10.1007/S11745-008-3217-Y/METRICS

Thakor, P., Mehta, J.B., Patel, R.R., Patel, D.D., Subramanian, R.B., Thakkar, V.R., 2016. Extraction and purification of phytol from *Abutilon indicum*: cytotoxic and apoptotic activity. *RSC Adv.* 6, 48336–48345. https://doi.org/10.1039/C5RA24464A

Tsuzuki, T., Tokuyama, Y., Igarashi, M., Miyazawa, T., 2004. Tumor growth suppression by α-eleostearic acid, a linolenic acid isomer with a conjugated triene system, via lipid peroxidation. *Carcinogenesis.* 25, 1417–1425. https://doi.org/10.1093/CARCIN/BGH109

Vahdati, S.N., Lashkari, A., Navasatli, S.A., Ardestani, S.K., Safavi, M., 2022. Butylated hydroxyl-toluene, 2,4-Di-tert-butylphenol, and phytol of *Chlorella* sp. protect the PC12 cell line against H_2O_2-induced neurotoxicity. *Biomedicine & Pharmacotherapy.* 145, 112415. https://doi.org/10.1016/J.BIOPHA.2021.112415

Vizetto-Duarte, C., Custódio, L., Gangadhar, K.N., Lago, J.H.G., Dias, C., Matos, A.M., Neng, N., Nogueira, J.M.F., Barreira, L., Albericio, F., Rauter, A.P., Varela, J., 2016. Isololiolide, a carotenoid metabolite isolated from the brown alga *Cystoseira tamariscifolia*, is cytotoxic and able to induce apoptosis in hepatocarcinoma cells through caspase-3 activation, decreased Bcl-2 levels, increased p53 expression and PARP cleavage. *Phytomedicine.* 23, 550–557. https://doi.org/10.1016/J.PHYMED.2016.02.008

Xu, W., Wang, J., Ju, B., Lan, X., Ying, X., Stien, D., 2022. Seven compounds from *Portulaca oleracea* L. and their anticholinesterase activities. *Nat Prod Res.* 36, 2547–2553. https://doi.org/10.1080/14786419.2021.1916928

Xuanji, X., Zengjun, G., Hui, Z., Xia, L., Jun, Luo, Dandan, L., Jun, Li, 2016. Chemical composition, *in vitro* antioxidant activity and α-glucosidase inhibitory effects of the essential oil and methanolic extract of *Elsholtzia densa* Benth. *Nat Prod Res.* 30, 2707–2711. https://doi.org/10.1080/14786419.2015.1135147

Yehia, R., Hathout, R.M., Attia, D.A., Elmazar, M.M., Mortada, N.D., 2017. Anti-tumor efficacy of an integrated methyl dihydrojasmonate transdermal microemulsion system targeting breast cancer cells: *in vitro* and *in vivo* studies. *Colloids Surf B Biointerfaces.* 155, 512–521. https://doi.org/10.1016/J.COLSURFB.2017.04.031

Younes, K.M., Romeilah, R.M., El-Beltagi, H.S., Moll, H. El, Rajendrasozhan, S., El-Shemy, H.A., Shalaby, E.A., 2021. *In-vitro* evaluation of antioxidant and antiradical potential of successive extracts, semi-purified fractions and biosynthesized silver nanoparticles of *Rumex vesicarius. Not Bot Horti Agrobot Cluj Napoca.* 49, 12293–12293. https://doi.org/10.15835/NBHA49112293

Zakaria, N.A., Ibrahim, D., Sulaiman, S.F., Supardy, N.A., 2011. Assessment of antioxidant activity, total phenolic content and *in vitro* toxicity of Malaysian red seaweed, *Acanthophora spicifera. J Chem Pharm Res.* 3, 182–191.

Zeisel, S.H., Da Costa, K.A., 2009. Choline: an essential nutrient for public health. *Nutr Rev.* 67, 615–623. https://doi.org/10.1111/J.1753-4887.2009.00246.X

Zeng, X., Tang, S., Dong, X., Dong, M., Shao, R., Liu, R., Li, T., Zhang, X., Wong, Y.H., Xie, Q., 2024. Analysis of metagenome and metabolome disclosed the mechanisms of *Dendrobium officinale* polysaccharide on DSS-induced ulcerative colitis-affected mice. *Int J Biol Macromol.* 277, 134229. https://doi.org/10.1016/J.IJBIOMAC.2024.134229

Zhao, J., Evangelopoulos, D., Bhakta, S., Gray, A.I., Seidel, V., 2014. Antitubercular activity of *Arctium lappa* and *Tussilago farfara* extracts and constituents. *J Ethnopharmacol.* 155, 796–800. https://doi.org/10.1016/J.JEP.2014.06.034